Tetrahedron Letters,Vol.28,No.43,pp 5169-5172,1987 0040-4039/87 $3.00 + .00
Printed in Great Britain Pergamon Journals Ltd.

REGIOSELECTIVE ENZYMIC HYDROLYSIS IN THE ISOLATION OF ISOMERS OF MUPIROCIN

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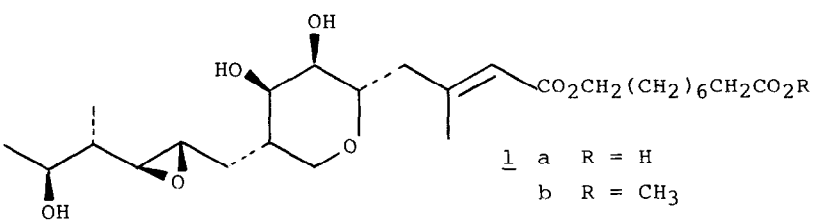
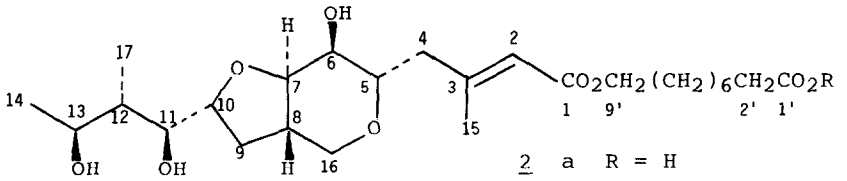
Abstract: The rearrangement isomers of pseudomonic acid A have been prepared using an enzyme catalysed, selective de-esterification, and full NMR interpretations of the acid salts have been determined.

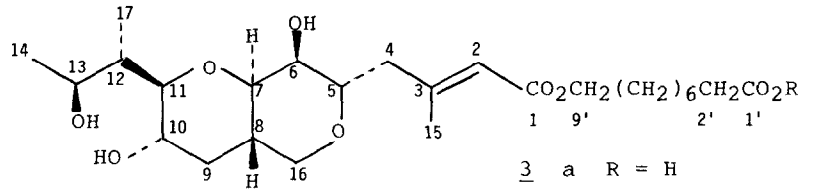
The acid and base catalysed rearrangement products of the marketed antibiotic pseudomonic acid A, 1a[1], have been shown to be the trans-fused bicyclic structures 2a and 3a[2]. Previous workers converted the mixture of acids obtained (2a and 3a) to the corresponding methyl esters (2b and 3b) prior to separation and absolute structure determination or, alternatively, the mixture of methyl esters could be obtained directly from methyl pseudomonate A, 1b[2]. Acid or base hydrolysis of the separated esters does not, however, give the original acids (2a and 3a), but is accompanied by hydrolysis of the activated allylic ester to release 9-hydroxynonanoic acid[2,3]; therefore the previous partial NMR assignments refer only to the methyl esters. In the search for an efficient route to the separate deprotected rearrangement isomers the selectivity offered by enzymes provided a promising approach. We wish to report a convenient, regioselective, enzymic hydrolysis of the esters 2b and 3b to provide the respective acids (2a and 3a) and sodium salts (2c and 3c) and full ^{1}H and ^{13}C NMR assignments of the latter.



1 a R = H
b R = CH_3



2 a R = H
b R = CH_3
c R = Na



3 a R = H
b R = CH_3
c R = Na

The methyl esters 2b and 3b were prepared from methyl pseudomonate A essentially by reported methodology[2]. Suspensions of these water insoluble esters in distilled water were hydrolysed with the commercially available protease, subtilisin Carlsberg (EC 3.4.21.14, ex Sigma), the mixture being maintained at pH 6.5 by the addition of 0.05M sodium hydroxide solution from an automatic titrator. Hence, the ester 2b (3.65g, 7.1mmol) was hydrolysed at ambient temperature in distilled water (100ml) with the protease (1440 units)[4] over 72 hours[5], after which the solution was freeze-dried to a white solid. This was re-dissolved in ethanol, filtered, and evaporated to give the sodium salt 2c as a white solid (3.49g, 6.6mmol). Although no organic impurities were detectable in this material by NMR or HPLC[6] a sample of this material was treated to remove any possible enzyme and inorganic salts present, by partitioning between ethyl acetate and water at 4°C with adjustment to pH 4 by addition of dilute hydrochloric acid. The organic phase on separation, yielded the acid 2a as a colourless oil[7]. This was dissolved in acetone and treated with sodium bicarbonate solution in water and the resultant solution at pH 7.5 freeze-dried to give a white solid. This was chromatographed on HP20SS resin[8] eluting with 5% THF/water and the eluent monitored by HPLC[6] to afford, after freeze-drying, the sodium salt 2c, mp 225°C (d).

Similarly the methyl ester 3b was converted, over 6 days, at ambient temperature to the sodium salt 3c and extensively purified *via* the acid[9] 3a to the salt 3c, mp. 138-139°C.

TABLE

NMR shifts in PPM, and assignments for D_2O solutions of the sodium salts 2c and 3c[10]

	^{13}C		^{1}H	
Carbon No.	2c	3c	2c	3c
1	169.08	168.95	-	-
2	118.46	118.50	5.81	5.83
3	158.24	158.14	-	-
4	40.76	39.94	2.66 eq, 2.36 ax	2.77 eq, 2.39 ax
5	77.98	78.04	4.18	4.21
6	69.36*	69.53	4.11	3.89
7	80.21	77.19	3.73	3.51
8	36.75	33.53	2.31	2.11
9	27.11	34.26	2.03 eq, 1.68 ax	2.04 eq, 1.22 ax
10	81.09	66.50	4.29	3.70
11	75.28	82.62	3.81	3.63
12	42.52	39.88	1.69	1.91
13	69.23*	70.53	4.14	3.84
14	17.90	21.22	1.12	1.25
15	18.78	18.82	2.14	2.17
16	66.11	64.85	4.02 eq, 3.68 ax	3.66 eq, 3.48 ax
17	10.54	9.65	0.85	0.94
1'	184.48	184.42	-	-
2'	38.61	38.66	2.16	2.18
3'	26.89	26.94	1.54	1.56
4'	29.83	29.90	1.41-1.28	1.44-1.27
5'	29.59	29.67		
6'	29.45	29.53		
7'	26.35	26.43		
8'	28.96	29.04	1.68	1.68
9'	65.69	65.69	4.15	4.16

* May be reversed

The complex ^{1}H-NMR spectra were assigned using a 2D COSY-45 experiment as well as conventional one dimensional (1D) decoupling experiments. The ^{13}C resonances of 2c and 3c were assigned using 1D experiments such as Spin-Echo and DEPT (both DEPT-90 and DEPT-135 variants) and 2D ^{1}H, ^{13}C COSY experiments which linked the carbon and proton resonances by one bond ($^{1}J_{CH}$) polarisation transfer. The TABLE shows the full NMR assignments for the sodium salts.

References and notes:

1. Approved generic name is mupirocin. The topical formulation is marketed by the Beecham Group under its Trade Mark BACTROBAN.
2. J.P. Clayton, R.S. Oliver, N.H. Rogers and T.J. King, J. Chem. Soc., Perkin I, 1979, 838.
3. J.P. Clayton, K. Luk and N.H. Rogers, J. Chem. Soc., Perkin I, 1979 308.
4. One unit is defined as the amount of enzyme which will hydrolyse casein to produce colour equivalent to 1.0μmol of tyrosine per minute at pH 7.5 at 37°C using Folin-Ciocalteu colour reagent.
5. As expected the rate of consumption of base was seen to increase on raising the temperature to 37°C giving the option of a shorter reaction time.
6. C_{18} Reverse phase column, eluting with 40% 0.05M ammonium acetate solution in methanol with UV detection at 240nm.
7. Characterised by ^{13}C-NMR. PPM (CD_3OD); 174.17, 168.11, 157.62, 118.63, 81.52, 81.19, 78.70, 76.71, 70.28, 70.05, 66.75, 64.90, 43.56, 41.74, 37.44, 34.95, 30.26, 30.16, 30.11, 29.77, 27.88, 27.04, 26.04, 19.05, 18.64, 11.29.
8. This highly porous polymer used to desalt the solution is a synthetic adsorbent and was obtained from Mitsubishi Chemical Industries Ltd.
9. Characterised by ^{13}C-NMR. PPM ($CDCl_3$); 177.76, 166.44, 155.06, 118.27, 82.07, 77.14, 76.13, 70.54, 68.97, 66.37, 64.29, 63.98, 40.08, 39.70, 34.58, 33.94, 32.90, 28.99, 28.90, 28.60, 28.56, 25.94, 24.71, 22.05, 18.41, 10.93.
10. All experiments were carried out on a Bruker AM400 NMR spectrometer, using external dioxan as a reference for the carbon-13 experiments, and external DSS for proton experiments.

(Received in UK 29 May 1987)